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Original Article

Phytochemical and antioxidant studies on leaves of *Costus malortieanus* H. Wendl.

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ABSTRACT

Present study aim at the screening of an unexplored plant, *Costus malortieanus* H.wendl.belonging to family Costaceae, commonly called stepladder ginger, which is used traditionally as anti diabetic and anti inflammatory. Total alcoholic extract of the leaves were subjected to antioxidant screening by DPPH, Iron chelating and nitric oxide methods. Estimation of phenolics, flavanoids and carotenoids were also carried out. Petroleum ether fraction of the ethanolic extract was analysed by GC-MS studies. The promising results of the different assays showed that the extract is a good source of antioxidants. GC-MS studies revealed the presence of antioxidant and antiinflammatory components in the extract.

Keywords: *Costus malortieanus*, DPPH, Nitric oxide, Iron chelating, flavanoids, phenolics, carotenoids, GC-MS.

INTRODUCTION

Nature is a unique source of phytochemicals possessing important biological activities, and the search for new lead compounds for developing novel pharmacological agents is increasing. The role of free radical reactions in disease pathology is well established and is known to be involved in many acute and chronic disorders in human beings, such as diabetes, atherosclerosis, aging, immunosuppression, and neurogeneration [1].

Costus malortieanus H. wendl. is a perennial herb of 2-3 feet height, commonly known as stepladder ginger (Fig.1). The genus *Costus* is a tropical genus with 42 species and is placed in the family Costaceae .Various *Costus* species have been used in traditional medicine in Europe and the Americas for a wide range of ailments [2].

Rhizome, root, stem and leaf of *Costus malortieanus* was found to contain diosgenin [3].The studies conducted to date have demonstrated that plants of the costus species have the potential to provide biologically active compounds, for the development of newer drugs. The purpose of the study is to screen the antioxidant activity of the ethanolic extract and its phytochemical analysis by GC-MS.

MATERIALS AND METHOD

***Plant material*:** Leaves of *Costus malortieanus* was collected from Calicut University and was identified by Mr. Rojimon.P. Thomas,Asst. Prof, CMS College, Kottayam (Herbarium Voucher specimen No.856). Fresh leaves were cut into small pieces and used for extraction.

***Extraction*:** Total ethanolic extract was prepared by hot maceration. Petroleum ether fraction of the extract was prepared and the yields were calculated. Preliminary phytochemical screening was carried out on the extracts. Total ethanolic extract was subjected to estimation of flavanoids,phenolics and carotenoids.

Total carotenoids: [4] The experiment was carried out in the dark to avoid photolysis of carotenoids once the saponification was complete. 0.025g extract was homogenized and saponified with 2.5 ml of 12% alcoholic potassium hydroxide in a water bath at 60^{O} C for 30 minutes. The saponified extract was treated with petroleum ether to collect the carotenoids. Small amount of anhydrous sodium sulphate was added to the petroleum ether extract to remove excess moisture. The final volume of petroleum ether extract was noted. Absorbance was measured at 450nm against blank. The amount of total carotenoids = (A450X volume

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of the sampleX100X4)/Weight of the sample. All results presented are mean ±SEM and were analysed in three replications.

Phenolic content [5]: The concentration of total phenolic content in the extracts was estimated by the Folin- Cio- Calteau method. 1 ml of (1mg/ml in methanol) solution of extracts were mixed with 5 ml of 2 N Folin Cio Calteau reagent(1:10 dilution) followed by 4 ml of sodium carbonate (7.5%w/v) after 5 minutes. The reaction mixture was incubated for 2 Hours at room temperature; the absorbance of the reaction mixture at 750 nm was measured against blank. The concentration of total phenolics in the extracts was expressed as mg gallic acid equivalent (GAE) per 100 g of dry weight using UV-Visible-Shimadzu-1601. All results presented are mean ±SEM and were analysed in three replications.

Flavanoid content [6]: Estimation of total flavanoid content was carried out by aluminium chloride method.1 ml of (1mg/ml in methanol) solution of extract and standard (rutin) were prepared. Different concentrations of the standard and 1mg of the sample solutions were taken and added 0.3ml of sodium nitrate solution. After 5 minutes 10% aluminium chloride (0.3ml) was added. At the 6^{th} minute, 1M sodium hydroxide (2ml) was added and made up the volume with distilled water. Solutions were mixed well and absorbance was measured at 510nm. All results presented are mean ±SEM and were analysed in three replications.

***Antioxidant activity*:**Antioxidant activity screening was conducted by the following models.

Iron chelating Assay [7]: Different concentrations of the extract, o-phenanthroline and 2M ferric chloride solution were mixed at ambient temperature for 10 minutes. After incubation, the absorbance of solution was measured at 510 nm against corresponding blank solution. The blank used here was a mixture of methanol and distilled water.

Nitric oxide Assay [8]: Reaction mixture containing sodium nitroprusside, phosphate buffer saline and sample solution was incubated at 25ºC for 2.5 hours. After incubation, 0.5 ml of the reaction mixture was mixed with one ml of sulphanilic acid reagent and allowed to stand for 5 minutes for completing diazotization. One ml of 1-naphthylamine was added, mixed and allowed to stand for 30 minutes at room temperature. A pink colour was formed in diffused light. A control was also prepared. The absorbances of the solutions were measured at 540nm against the corresponding blank solution. The percentage inhibition was calculated for the samples and standard using the following equation.

% inhibition= [absorbance of control- absorbance of test]/absorbance of control.

DPPH Assay [9]:1 ml of Methanolic DPPH solution was added to 3 ml of extract at different concentrations. 30minutes later, the absorbance was read at 517nm. The experiments were performed in triplicate. Radical scavenging activity was calculated by the formula, percentage inhibition= (Absorbance of blank-sample)/absorbance of blank. All samples were assayed in triplicate and averaged. The concentration of the extract required to scavenge 50% of the radicals was calculated by plotting a graph of percentage inhibition versus concentration, in all the antioxidant assays. Results are depicted in table 2.

GC-MS analysis of Petroleum ether fraction of ethanolic extract of C. malortiaenus.

The instrument used is Variant CP-300 saturn 2200 GC/MS/MS with factor four VF-5 MF column. Oven temperature was maintained at 100ºC for 1.5 minutes and gradually increased to 270ºC at 5ºC per minute. One microlitre sample was injected for analysis. Helium gas 99.9% pure was used as the carrier gas; the flow rate of carrier gas was 1ml/minute. Sample injected temperature was maintained at 250ºC and split ratio is 20 throughout the experiment period. The ionization mass was done with 70eV. The mass spectrum was recorded for the mass range 140-600 m/z for 60 minutes. Compound separated on elution through column were detected in electronic signals. The m/z ratio obtained was calibrated through graph obtained which was called as the mass spectrum graph which is the finger print of the molecule. The identification of the compound was based on the comparison of their mass spectra with NIST library and is tabulated in Table No.3.

***Statistical Analysis*:** All the data expressed were presented as mean values ± SEM. All experiments were carried out in triplicate. IC_{50} values were found out using excel 2007.

RESULT AND DISCUSSION

Extraction of leaves of *C. malortiaenus* with 95% ethyl alcohol yielded 3.27% w/w and the petroleum ether fraction of the ethanolic extract was found to be 0.801 % w/w. Preliminary phytochemical screening showed the presence of terpenoids, phenolics and flavanoids in the ethanolic extract. Estimations of Phenolics, flavanoids and carotenoids were carried out on the ethanolic extract and depicted in Table no.1.

Nitric oxide is an important chemical mediator, involved in number of physiological actions and regulation of cell mediated toxicity. Excess of nitric oxide react with oxygen and generate nitrite and peroxy nitrite anions, which can act as free radicals, resulting in various deleterious effects in the cells. So the production of nitric oxide should be regulated as much as possible [10]. The extract inhibits nitrite formation by directly competing with oxygen in the reaction with nitric oxide [8]. IC_{50} value of the ethanolic extract was found to be 839.47µg/ml and that of ascorbic acid was 49.77 µg/ml. The result indicates moderate nitric oxide inhibition by the extract.

DPPH Assay is one of the most popular methods in natural antioxidant studies [11]. This method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form DPPH-H by the reaction [12]. The extract has the ability to reduce DPPH to the yellow coloured diphenyl picryl hydrazine. The IC_{50} value of the extract was found to be 419.1µg/ml and that of ascorbic acid was 5.5 µg/ml. The result shows better inhibition by DPPH assay than nitric oxide model.

Iron is essential for life because it is required for oxygen transport, respiration and activity of many enzymes. However, iron is an extremely reactive metal and catalyzes oxidative changes in lipids, proteins and other cellular components [13,14]. It causes lipid peroxidation through the Fenton and Haber-weiss reaction and decomposes the lipid hydroxide into peroxyl and Alkoxyl radicals that can perpetuate the chain reactions [15]. Ortho substituted phenolic compounds may exert pro-oxidant effects by interaction with iron. The principle behind Iron chelating assay is that the ferric ions are reduced in presence of antioxidants to ferrous ions. O-phenanthroline forms chelates with ferrous ions to form orange red complex whose intensity is measured at 510 nm. In this method, the IC_{50} value of ethanolic extract was found to be 99.80µg/ml and that of ascorbic acid was 10.75µg/ml. The result indicates better inhibition by iron chelating assay than the other models.

GC-MS studies showed the presence of antioxidant and anti-inflammatory components ie, cucurbitacin B, Lupeol and Friedelan-3-one, in the petroleum ether fraction. This shows the medicinal value of the extract. The antioxidant components may be responsible for the activity shown by the iron chelating, DPPH and nitric oxide assays. The extract may be having anti-inflammatory activity due to the presence of above compounds. Other components prevailing are 2-Ethylthiolane, S,S-dioxide, Styrene, copaene, gamma murrolene and beta cubebene (Figure.2).

It is well known that there is a strong relationship between total phenol content and antioxidant activity, as phenols possess strong scavenging ability for free donors because electron delocalisation across the molecule efficiently stabilises the resulting phenoxy radicals [16]. Flavanoids like many other polyphenols are excellent free radical scavengers because they are highly reactive as hydrogen or electron donors [17].Therefore, the phenolic and flavanoid content of plants may directly contribute to their antioxidant action.

CONCLUSION

The present study showed the significant antioxidant potential of *C. malortiaenus* by *in vitro* assays. It was found that the extract of *C. malortiaenus* might be helpful in preventing the progress of various oxidative stresses. Therefore, further studies are in progress to investigate the underlying mechanism behind and to isolate the components responsible for the activity.

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Table.1 Estimation of phenolics,flavanoids and carotenoids.

Flavanoids		Phenolics		Carotenoids	
Mean±SEM	**Rutin equivalent µg/100g**	**Mean ±SEM**	**Gallic acid equivalent µg/100g**	**Mean±SEM**	**µg/100g**
0.032±0.0008	**45.00**	**0.16±0.0011**	**15.00**	**0.537±0.00176**	**8608**

Values are equivalent to mcg/100g extract.
Values expressed are mean± SEM; n=3

Table 2.Percentage Inhibition of Standard and Extracts by Antioxidant assays

Method	Extracts	Concentration(mcg/ml)	Absorbance±SEM	% Inhibition
Nitric oxide	Standard Ascorbic acid	Control 20 40 60 80 100	0.912±0.001 0.537±0.002 0.479±0.001 0.432±0.001 0.383±0.0005 0.305±0.002	- 41.11 47.47 52.63 58.00 66.55
	C.m	Control 125 250 500 750 1000	0.243±0.0003 0.203±0.0021 0.169±0.0020 0.136±0.0020 0.128±0.001 0.115±0.0025	- 16.46 30.45 44.03 47.32 52.67
DPPH	Standard Ascorbic acid	Control 2 4 6 8 10	0.72±0.00321 0.49±0.0018 0.41±0.0012 0.353±0.0044 0.263±0.0023 0.192±0.0017	- 31.94 43.06 50.97 63.40 73.12
	C.m	Control 200 300 400 500 600	0.334±0.005 0.2303±0.0008 0.1966±0.0021 0.1730±0.0036 0.1426±0.1135 0.1217±0.0008	- 31.04 41.13 48.20 57.30 63.56
Iron chelating	Standard Ascorbic acid	Control 5 10 15 20	0.022±0.0011 0.030±0.0011 0.041±0.0013 0.074±0.0017 0.140±0.0017	- 26.66 46.34 70.27 84.28
	C.m	Control 25 50 100 250 500	0.041±0.0030 0.0536±0.0008 0.0713±0.0003 0.1240±0.0051 0.1866±0.0054 0.2870±0.0014	- 23.5 42.49 66.935 78.05 85.71

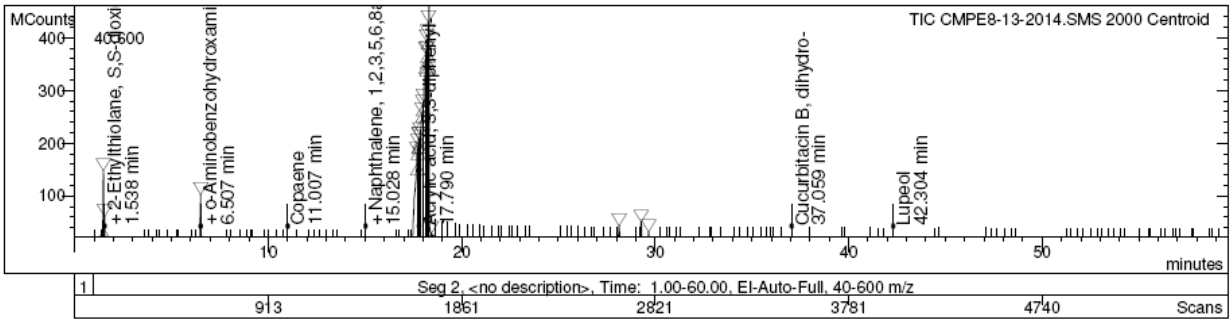
Values expressed are mean±SEM, n=3.C.m is ethanolic extract of C.malortieanus.

Table 3. Compounds identified by GC-MS analysis of Petroleum ether fraction of ethanolic extract of C. *malortiaenus* leaves.

Name of compound	% Area	RT
2-Ethylthiolane, S,S-dioxide	**3.364**	1.538
Styrene	**1.885**	2.378
Carbamic acid,N-methyl-,phenyl ester		2.944
2-Dodecanol	-	3.165
N-[3-[N-Aziridyl] propylidine] furfurylamine		4.408
Cyclohexanol, 2-methyl-3-(1-methylethenyl)	0.063	5.655
o-Aminobenzohydroxamic acid	0.0254	6.503
Ethyl salicylate	0.0252	8.207
Copaene	**6.202**	11.007
Gamma murrolene	**2.222**	13.724
Beta Cubebene	**1.042**	13.934
Delta Cadinene	0.2888	15.013
Acrylic acid,3,3-diphenyl	0.7229	17.796
Methyl 15-methyl Palmitate	0.81154	30.122
Butyl isobutyl phthalate	**2.960**	30.445
Cucurbitacin B, dihydro	-	37.188
Benzene,1,1'-[2-methyl-2-(phenylthio)cyclopropylidene]	-	39.918 -
Carnegine	0.61775	40.449
Lupeol	-	42.273
Friedelan-3-one	-	49.496

Figure:1. *Costus malortieanus*

Figure: 2. GC-MS spectrum of Petroleum ether fraction of ethanolic extract of *C. malortieanus* leaves



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